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Diagnostic value of enzyme-linked immunosorbent assays using hypothetical proteins CT226 and CT795 as antigens in *Chlamydia trachomatis* serodiagnosis

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Abstract

The CT226 and the CT795 proteins were produced as purified recombinant proteins and were used as antigens in enzyme-linked immunosorbent assay (ELISA) tests for the detection of *Chlamydia trachomatis* IgG antibodies. The performances of the developed ELISA tests were compared with our in-house microimmunofluorescence test and the species-specific pELISA test using a panel of 342 sera. Our results indicate that the performance of the CT795 ELISA test was higher than that of the CT226 ELISA test according to the microimmunofluorescence and to the pELISA. To assess whether a combination of tests could improve the serodiagnosis of *C. trachomatis* infections, we associated results obtained with these tests to that using the previously developed CT694 ELISA test. Combining ELISA test results did not improve significantly the performances of these ELISA tests. The CT795 ELISA test, showing the highest performance, may be used for the serodiagnosis of *C. trachomatis* infections.

Keywords: *Chlamydia trachomatis*; Hypothetical protein; Expression vector; MIF; CT226 ELISA; CT694 ELISA; CT795 ELISA

1. Introduction

Genital *Chlamydia trachomatis* infections have been recognized as the most prevalent bacterial sexually transmitted infections (STIs) throughout the world. Different tests have been used for chlamydial serology. The microimmunofluorescence (MIF) test was considered for many years the gold standard in *C. trachomatis* serodiagnosis. Although it was claimed to be species specific, cross-reactions between chlamydial species have been reported (Frikha-Gargouri et al., 2008a; Gijsen et al., 2001; Moss et al., 1993; Wagenvoort et al., 1999). Thus, several *C. trachomatis* species-specific enzyme-linked immunosorbent assays (ELISAs) have been commercially developed for serodiagnosis using synthetic peptides as antigens. Among the different antigens used to detect antibodies to *C. trachomatis*, large differences in sensitivity and specificity can be observed (Land et al., 2003). The pELISA test (Medac, Hamburg, Germany) was found previously to have the best diagnostic value in the serodiagnosis of *C. trachomatis* infection (Land et al., 2003). In 2006, Sharma et al. (2006) identified 7 chlamydial antigens as relatively immunodominant in an assay using recombinant fusion proteins, including the major outer membrane protein (MOMP) encoded by open reading frame (ORF) CT681. The remaining antigens were encoded by the hypothetical ORFs CT089, CT147, CT226, CT694, CT795, and CT858. We recently investigated the performances of the CT694 ELISA test according to the MIF test and to the pELISA test (Frikha-Gargouri et al., 2009). A high performance of the developed ELISA test was noted according to the pELISA test (Frikha-Gargouri et al., 2009). Because the CT226 and the CT795 proteins had not been compared with other *C. trachomatis* antigens, we investigated whether their use in ELISA was sensitive and specific according to the MIF test and to the pELISA test.

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Furthermore, we investigated whether a test or a combination of tests, using the CT226, the CT795, as well as the CT694 as antigens, could be sensitive and specific enough to be used for the serodiagnosis of *C. trachomatis* infection. The 3 recombinant protein-based ELISA tests were prepared and tested under exactly the same conditions to compare their respective sensitivities and specificities.

2. Materials and methods

2.1. Serum samples

Different collection of sera were used in this study : i) 50 sera collected from children, aged 2 to 8 years, who were *C. trachomatis* and *Chlamydophila pneumoniae* IgG MIF negative; ii) 49 sera collected from patients who were MIF positive to only *C. pneumoniae* antigen; iii) 22 sera collected from patients who were MIF positive to only *C. trachomatis* antigen; iv) 40 sera obtained from patients attending STI clinics suspected to have chlamydial infections diagnosed by Cobas Amplicor (Roche Diagnostics, Basel, Switzerland) test as polymerase chain reaction (PCR) positive (n = 13) or PCR negative (n = 27); v) 72 sera were from a high-risk population of prostitutes; and vi) 109 sera collected from Tunisian healthy blood donors (HBDs). All subjects provided verbal informed consent, and the study protocol was approved by our ethics committee (Association d'Enregistrement et de Lutte Contre le Cancer du Sud Tunisien).

2.2. Determination of percentage identities between the CT226 and the CT795 proteins of C. trachomatis serovar D with those of the other serovars

The CT226 and CT795 protein sequences of *C. trachomatis* serovar D were retrieved from the National Center for Biotechnology Information (NCBI) database in FASTA format. The BLASTP program was used to search proteins presenting homology with these proteins of *C. trachomatis* serovar D in the chlamydial species infecting humans and to determine the percentage identities between the different proteins (http://www.ncbi.nlm.nih.gov/blast/). Accession numbers of protein sequences used are listed in Table 1.

2.3. Generation of the recombinant protein

All the experimental conditions performed to generate the recombinant proteins, CT226 and CT795, were identical to those reported previously (Frikha-Gargouri et al., 2008b, 2009). Briefly, *C. trachomatis* serovar D DNA was extracted using an in-house method based on proteinase K treatment. The gene encoding the full-length CT226 protein sequence was amplified by PCR using the primers 5′ GCTAGGATCCATGTTGGCCTTTTTTTTGCGAA 3′ and 5′ TAGCGCGGCCGCTTATATCAGACTTTCTTCCAATAC 3′, whereas the gene encoding the full-length CT795 protein sequence was amplified by PCR using the primers 5′ GCTAGGATCCATGAGATTCTTGTTAGCTTTATTC 3′ and 5′ TAGCGCGGCCGCCTACTCAACAAATTCAGGATTTA 3′. These 2 PCR fragments were then cloned into the pGEX-6P-1 expression vector (Amersham Biosciences, Uppsala, Sweden) harboring the Glutathione S transferase (GST) as a tag. DNA sequences of the inserts of the recombinant plasmids were elucidated by the dideoxynucleotide chain termination method. The recombinant plasmids were then used to transform *Escherichia coli* strain BL21 (DE3) competent cells. The CT226 and CT795 recombinant proteins were overexpressed in *E. coli* BL21 (DE3) after induction with 1 mmol/L isopropyl-β-D-thiogalactopyranoside for 3 h at 37 °C. The recombinant proteins were then purified using glutathione bead affinity chromatography. Purified protein concentrations were determined as described by Bradford (1976) using bovine serum albumin (BSA) as a standard. Polyacrylamide gel electrophoresis (PAGE) was performed in the presence of sodium dodecyl sulphate (SDS) (0.3 mol/L) and β-mercaptoethanol (0.25 mol/L) as described by Laemmli (1970).

2.4. Serologic methods

All sera were tested using the MIF, the pELISA, and the developed CT226 and CT795 ELISA tests.

2.4.1. Microimmunofluorescence test

The MIF test was performed by standard procedures as an in-house method (Frikha-Gargouri et al., 2008b). Purified elementary bodies of *C. pneumoniae* IOL-207 strain, *Chlamydia psittaci* Loth strain, and *C. trachomatis* L2 strain were

Table 1
Percentage identity determination between the CT226 and the CT795 proteins within the chlamydial species

Chlamydial species	Serovars	CT226 protein		CT795 protein	
		Accession no.	Identity	Accession no.	Identity
C. trachomatis	A	YP_328034	91.5% (161/176)	YP_328624	100.0% (163/163)
	D	NP_219731	100.0% (176/176)	NP_220315	100.0% (163/163)
	L2	YP_001654555	94.9% (167/176)	YP_001654255	98.8% (161/163)
	L2b	YP_001653567	94.9% (167/176)	YP_001653267	98.8% (161/163)
C. pneumoniae CWL029		NP_225140	0.0% (0/176)	NP_225140	27.2% (55/202)
C. psittaci		_[a]	_[a]	_[a]	_[a]

[a] No sequence homology of *C. psittaci* encoding proteins with the CT226 and the CT795 proteins of *C. trachomatis* were identified by the BlastP program.